PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference WPP286912	FOR FURTHER ACTION	See Form PCT/IPEA/416
International application No. PCT/GB2004/000768	International filing date *(day/month/year)* 26.02.2004	Priority date *(day/month/year)* 26.02.2003
International Patent Classification (IPC) or national classification and IPC C12P21/08, C12N5/16, C12N15/85, C12N15/13, A01K67/027, C07K16/00		
Applicant BABRAHAM INSTITUTE et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 6 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 11 sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☒	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
09.12.2004	01.03.2005
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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/GB2004/000768

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-54 as originally filed

Sequence listings part of the description, Pages

1-5 as originally filed

Claims, Numbers

1-72 filed with telefax on 09.12.2004

Drawings, Sheets

1-18 as originally filed

☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed

   ☒ filed together with the international application in computer readable form

   ☐ furnished subsequently to this Authority for the purposes of search and/or examination

   ☐ received by this Authority as an amendment on

2. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional observations, if necessary:

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/GB2004/000768

Box No. II Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:
   - ☐ copy of the earlier application whose priority has been claimed (Rule 66.7(a)).
   - ☐ translation of the earlier application whose priority has been claimed (Rule 66.7(b)).
2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rule 64.1). Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.
3. Additional observations, if necessary:

   see separate sheet

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-72
	No:	Claims	none
Inventive step (IS)	Yes:	Claims	1-72
	No:	Claims	none
Industrial applicability (IA)	Yes:	Claims	1-72
	No:	Claims	none

2. Citations and explanations (Rule 70.7):

   see separate sheet

Re Item II

Priority

The priority document is available (received on 27 September 2004 at the EPO). The priority appears to be validly claimed. Consequently, the document US6,570,061 cited in the International Search Report as a P,X document will not be considered for the establishment of the following opinion.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following documents:

D1: WO 98/54348 A (BRUGGEMANN MARIANNE ; BABRAHAM INST (GB)) 3 December 1998

D2: ZOU YONG-RUI ET AL: "Cre-loxP-mediated gene replacement: A mouse strain producing humanized antibodies" CURRENT BIOLOGY, vol. 4, no. 12, 1994, pages 1099-1103

D3: METZGER D ET AL: "CONDITIONAL SITE-SPECIFIC RECOMBINATION IN MAMMALIAN CELLS USING A LIGAND-DEPENDENT CHIMERIC CRE RECOMBINASE" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 92, no. 15, 18 July 1995, pages 6991-6995

D4: WO 90/04036 A (AGRICULTURAL & FOOD RES ; BRUGGEMANN MARIANNE (GB); MEDICAL RES COUNCI) 19 April 1990

2. Novelty

D1 discloses mice wherein the telomeres comprising the genes encoding the immunoglobulin heavy chains have been deleted, leading to deletion of the constant and variable regions of the immonoglobulin heavy chain and replacement by the human immunoglobulin heavy chain locus (see Figure 3).

D2 discloses a method for replacement of one gene of the

None of the available documents discloses an animal wherein the chromosome fragment encoding the constant region of the immunoglobulin heavy chain is absent but wherein at least some segments of the variable region of the immunoglobulin heavy chain are present, in view of obtaining animals producing humanized immunoglobulins comprising a full immunoglobulin heavy chain constant region of human origin.

Consequently, it is considered that the subject-matter of **claims 1-72** meets the requirements of Art. 33(2) PCT concerning novelty.

3. Inventive step

D1, **D2** and **D4** are concerned with the production of transgenic animals wherein parts of the immunoglobulin heavy chain region have been deleted and replaced by the corresponding human parts of the immunoglobulin heavy chain regions in order for the animal to produce humanized immunoglobulins.

In **D1**, the complete mouse variable and constants immunoglobulin heavy chain regions are replaced with the human variable or constant immunoglobulin heavy chain region.

In **D2**, the Cγ1 gene of the immunoglobulin heavy chain constant region is replaced by its human counterpart using the CRE-Lox recombination technique.

In **D4**, mouse immunoglobulin constant regions are prefernetially replaced with their human counterparts (see page 4, lines 16-24).

However, none of the documents suggests that the entire IgH region should be deleted (for eventually being replaced by its human counterpart).

Consequently, it is considered that the subject-matter of **claims 1-72** meets the requirements of Art. 33(2) PCT concerning inventive step.

Claims:

1. A genetically modified non-human mammal or cell characterised in that it does not comprise a nucleic acid sequence which itself encodes any endogenous immunoglobulin heavy chain constant region locus polypeptide and in that one or more endogenous Ig H Variable region, one or more endogenous Ig H D segment, and one or more endogenous Ig H J segment nucleic acid sequences are present.

2. A genetically modified non-human mammal or cell characterised in that it does not comprise a nucleic acid sequence which itself encodes any endogenous immunoglobulin heavy chain constant region locus polypeptide and in that all the endogenous Ig H Variable region, D and J segment nucleic acid sequences are present.

3. A genetically modified non-human mammal or cell according to claim 1 or claim 2 characterised in that it does not comprise a nucleic acid sequence which itself encodes any immunoglobulin heavy chain constant region (IgH C) polypeptide.

4. A genetically modified non-human mammal or cell according to any of claims 1 to 3 characterised in that all immunoglobulin heavy chain constant region gene sequences are absent or partially absent from the genome.

5. A genetically modified non-human mammal or cell according to any of the preceding claims, characterised in that it is obtainable or obtained by targeted deletion of essentially all endogenous IgH C gene sequences.

6. A genetically modified non-human mammal or cell according to any of the preceding claims characterised in that it is obtainable or obtained by Cre *loxP* recombination.

7. A genetically modified non-human mammal or cell according to any of the preceding claims characterised in that at least part of at least one IgH C gene enhancer sequence is present.

8. A genetically modified non-human mammal or cell according to any of the preceding claims characterised in that a non-endogenous site-specific recombination sequence is present within the genome.

9. A genetically modified non-human mammal or cell characterised by having a non-endogenous site-specific recombination sequence downstream of, or within the last gene of the IgH C locus.

10. A genetically modified non-human mammal or cell according to claim 8 characterised by having a further non-endogenous site specific recombination sequence upstream of, or within the first gene of the IgH C locus.

11. A genetically modified non-human mammal or cell according to any of the preceding claims characterised in that one or more selectable marker(s) is present within the genome.

12. A genetically modified non-human mammal or cell according to claim 8 characterised in that at least one selectable marker is present upstream of, or downstream of, the non-endogenous site specific recombination sequence.

13. A genetically modified non-human mammal or cell according to claim 9 characterised in that at least one selectable marker is integrated within the genome upstream of, and/or downstream of, at least one non-endogenous site specific recombination sequence.

14. A genetically modified non-human mammal or cell according to any of claims 11 to 13 characterised in that the selectable marker(s) is one or more selectable marker selected from a group comprising a neomycin resistance gene, a puromycin resistance gene, and a hygromycin resistance gene.

15. A genetically modified non-human mammal or cell according to any of claims 7 to 14 characterised in that the non-endogenous site-specific recombination sequence is a *lox*P site.

16. A genetically modified non-human mammal according to any of the preceding claims characterised in that it is a mouse.

17. A genetically modified non-human cell according to any of claims 1 to 15 characterised in that it is a mouse cell.

18. A genetically modified mouse according to claim 16, or a genetically modified mouse cell according to claim 17, characterised in that all eight endogenous IgH C genes μ, δ, γ3, γ1, γ2a, γ2b, ε and α are absent or partially absent.

19. A genetically modified non-human cell according to any of claims 1 to 15 or claim 17 or 18 characterised in that it is an embryonic stem cell.

20. A genetically modified non-human mammal derived from a genetically modified non-human mammal of any of claims 1 to 16 or claim 18.

21. A genetically modified non-human mammal derived from a genetically modified non-human cell of any of claims 1 to 15 or any of claims 17 to 19.

22. A genetically modified non-human cell derived from a genetically modified non-human mammal of any of claims 1 to 16 or claim 18.

23. A method for producing a genetically modified non-human cell comprising:

(a) (i) transfecting a non-human cell with a targeting construct for integration upstream of, or within the first IgH C gene of the IgH C locus, said targeting construct comprising a non-endogenous site specific recombination sequence and a selectable marker, selecting for a cell in

which the selectable marker is present and screening said cell for integration of the recombination sequence, and,

(ii) transfecting a cell produced in (a)(i) with a targeting construct for integration downstream of, or within the last IgH C gene of the IgH C locus, said targeting construct comprising a selectable marker and a non-endogenous site-specific recombination sequence, selecting for a cell in which the selectable marker is present and screening said cell for integration of the recombination sequence; or

(b) (i) transfecting a non-human cell with a targeting construct for integration downstream of, or within the last IgH C gene of the IgH C locus, said targeting construct comprising a non-endogenous site-specific recombination sequence and a selectable marker selecting for a cell in which the selectable marker is present, and screening said cell for integration of the recombination sequence, and

(ii) transfecting a cell produced in (b)(i) with a targeting construct for integration upstream of, or within the first IgH C gene of the IgH C locus, said targeting construct comprising a non-endogenous site-specific recombination sequence and a selectable marker, selecting for a cell in which the selectable marker is present, and screening said cell for integration of the recombination sequence; or

(c) co-transfecting a non-human cell with a targeting construct for integration upstream of, or within the first IgH C gene of the IgH C locus and with a targeting construct for integration downstream of, or within the last IgH C gene of the IgH C locus, each of said targeting constructs comprising a non-endogenous site specific recombination sequence and each having a selectable marker, selecting for a cell in which the selectable marker(s) is/are present, and screening said cell for integration of the recombination sequence; and optionally,

(d) providing to a cell obtained in (a)(ii), (b)(ii) or (c) a recombinase active at the non-endogenous site-specific recombination sequence and, optionally, screening for deletion events.

24. A method according to claim 23 characterised in that the non-endogenous site-specific recombination sequence is a *lox*P site.

25. A method according to claim 24 characterised in that, in optional step (d), the recombinase is a Cre recombinase.

26. A method according to any of claims 23 to claim 25 characterised in that the recombinase is provided by an expression vector.

27. A method according to any of claims 23 to 26 characterised in that the genetically modified non-human cell is a mouse cell.

28. A method according to any of claims 23 to 27 characterised in that the genetically modified non-human cell is an embryonic stem cell.

29. The use of an embryonic stem cell of claim 19 or a cell obtainable by a method of any of claims 23 to 28 for the production of a genetically modified non-human mammal.

30. A method for producing a genetically modified non-human mammal characterised in that an embryonic stem cell of claim 19 or obtainable by a method of claim 28 is introduced into a host blastocyst and developed into a chimaeric animal.

31. A method according to claim 30 characterised by:

(a) introducing a non-human mammal embryonic stem cell according to claim 19 or obtainable by a method of claim 28 into a compatible non-human mammal blastocyst, and

(b) transplanting the blastocyst obtained in (a) into a compatible non-human mammal foster mother to obtain a chimaeric non-human mammal, and optionally, screening for the selectable marker(s), and/or the non-endogenous site specific recombination sequence(s), and/or for deletion of essentially all endogenous IgH C gene sequences.

32. A method for producing a genetically modified non-human mammal characterised in that the chimaeric non-human mammal according to claim 30 or claim 31 is bred to obtain heterozygous progeny.

33. A method for producing a genetically modified non-human mammal characterised in that the heterozygous progeny of claim 32 is inter-bred to obtain homozygous progeny.

34. A method for producing a genetically modified non-human mammal characterised by cross-breeding a genetically modified non-human mammal homozygous for integration of a non-endogenous site-specific recombination sequence upstream of, or within the first IgH C gene of the IgH C locus with a compatible genetically modified non-human mammal homozygous for integration of a non-endogenous site-specific recombination sequence downstream, or within the last IgH C gene of the IgH C locus, to obtain heterozygous progeny and optionally interbreeding the heterozygous progeny to obtain progeny homozygous for both integrations.

35. A method according to claim 34 characterised by further comprising cross-breeding progeny homozygous for both integrations with a compatible non-human mammal capable of expressing a recombinase active at the non-endogenous site specific recombination sequence to obtain progeny; and optionally screening the progeny obtained for IgH C gene deletion.

36. A method according to claim 34 or claim 35 characterised in that the non-endogenous site specific recombination sequence(s) are *lox*P sites.

37. A method according to claim 36 characterised in that the recombinase is a Cre recombinase.

38. A method according to claim 36 characterised by further comprising cross-breeding progeny heterozygous or homozygous for *lox*P at both loci with a compatible non-human mammal capable of expressing Cre recombinase to obtain a progeny non-human mammal that does not comprise a nucleic acid sequence

which itself encodes any endogenous Ig heavy chain constant region polypeptide on one or both alleles.

39. A genetically modified non-human mammal characterised in that it is obtainable or obtained by a method of claim 35 to claim 38 and does not comprise a nucleic acid sequence which itself encodes any endogenous Ig heavy chain constant region polypeptide and in that one or more endogenous Ig H Variable region, one or more endogenous Ig H D segment, and one or more endogenous Ig H J segment nucleic acid sequences are present.

40. A genetically modified non-human mammal characterised in that it is obtainable or obtained by a method of claim 35 to claim 39 and does not comprise a nucleic acid sequence which itself encodes any endogenous Ig heavy chain constant region polypeptide and that all the endogenous Ig H Variable region, D and J segment nucleic acid sequences are present.

41. A method for producing a genetically modified non-human mammal capable of expressing one or more exogenous genes, characterised by breeding a genetically modified non-human mammal according to claims 1 to 7 or claims 10 to 16 or claims 18 to 21 that does not comprise a nucleic acid sequence which itself encodes any endogenous immunoglobulin heavy chain constant region polypeptide, with a compatible non-human mammal that encodes and is capable of expressing one or more exogenous gene(s), to obtain progeny heterozygous for the one or more exogenous gene(s), and optionally inter-breeding the heterozygous progeny to produce progeny homozygous for the one or more exogenous gene(s).

42. A method for producing a genetically modified non-human mammal or cell capable of expressing one or more exogenous gene(s) characterised by comprising introduction of one or more exogenous gene(s) into a non-human mammalian cell according to claims 1 to 7 or claims 10 to 15 or claims 17 to 21 that does not comprise a nucleic acid sequence which itself encodes any endogenous immunoglobulin heavy chain constant region polypeptide.

43. A method according to claim 42 characterised in that the non-human mammalian cell is an embryonic stem cell.

44. A method according to claim 43, characterised in that the one or more exogenous gene(s) are introduced by transfection.

45. A method according to claim 42 characterised in that the non-human mammal cell is an oocyte (egg cell).

46. A method according to claim 45, characterised in that the one or more exogenous gene(s) are introduced by DNA micro-injection.

47. A method according to any of claims 42 to 46 characterised in that the one or more exogenous gene(s) are inserted into the genome of the non-human mammal or cell.

48. A method according to claim 47 characterised in that the one or more exogenous gene(s) are inserted into a non-endogenous site specific recombination sequence.

49. A method for producing a genetically modified non-human mammal capable of expressing one or more exogenous gene(s) characterised by cross-breeding a non-human mammal that does not comprise a nucleic acid sequence which itself encodes any endogenous immunoglobulin heavy chain constant region polypeptide and in that one or more endogenous Ig H Variable region, one or more endogenous Ig H D segment, and one or more endogenous Ig H J segment nucleic acid sequences are present with a transgenic mammal having one or more exogenous gene(s) associated with or flanked by a non-endogenous site specific recombination sequence and having a recombinase active at the non-endogenous site specific recombination sequence to obtain progeny and optionally screening the progeny for insertion of the one or more exogenous gene(s).

50. A method for producing a genetically modified non-human mammal capable of expressing one or more exogenous gene(s) characterised by cross-

breeding a non-human mammal that does not comprise a nucleic acid sequence which itself encodes any endogenous immunoglobulin heavy chain constant region polypeptide and in that all the endogenous Ig H Variable region, D and J segment nucleic acid sequences are present.with a transgenic mammal having one or more exogenous gene(s) associated with or flanked by a non-endogenous site specific recombination sequence and having a recombinase active at the non-endogenous site specific recombination sequence to obtain progeny and optionally screening the progeny for insertion of the one or more exogenous gene(s).

51. A method according to any of claims 46 to 50 characterised in that the non-endogenous site specific recombination sequence is a *loxP* sequence and insertion is by Cre – *lox P* integration.

52. A method according to any of claims 41 to 51 characterised in that the genetically modified non-human mammal is a mouse.

53. A method according to any of claims 41 to 52 characterised in that the exogenous gene or genes is an Ig H gene or Ig H genes.

54. A method according to claim 53 characterised in that the Ig H gene or genes is an IgH C gene or IgH C genes.

55. A method according to any of claims 41 to 54 characterised in that the exogenous genes or genes are a human gene or human genes.

56. A method according to any one of claims 41 to 55 characterised in that the exogenous genes are a human Ig heavy chain locus having V, D, J and/or C regions.

57. A method according to claim 56 wherein the human Ig heavy chain locus V, D, J and/or C regions are in germline configuration.

58. A method according to claim 56 wherein the human Ig heavy chain locus V, D, J and/or C regions are productively arranged.

59. A non-human mammal or cell obtainable by a method of any of claims 41 to 58.

60. The use of a non-human mammal or cell according to claim 59 in the production of an exogenous immunoglobulin.

61. The use of a non-human mammal or cell according to claim 59 in the production of a human immunoglobulin.

62. A method for production of exogenous immunoglobulin comprising use of a non-human mammal or cell according to claim 59.

63. A method for production of human immunoglobulin comprising use of a non-human mammal or cell according to claim 59.

64. A method or use according to any one of claims 60 to 63 wherein the non-human mammal is a rodent.

65. A method or use according to any one of claims 60 to 63 wherein the non-human mammal is a mouse.

66. A method or use according to any one of claims 60 to 63 wherein the non-human cell is a rodent cell.

67. A method or use according to any one of claims 60 to 63 wherein the non-human cell is a mouse cell.

68. An immunoglobulin obtainable or obtained by a method according to any one of claims 62 to 67.

69. A human immunoglobulin obtainable or obtained by a method according to any one of claims 62 to 67.

70. An immunoglobulin according to claim 68 or claim 69 for use as a medicament.

71. The use of an immunoglobulin according to claim 68 or claim 69 in the manufacture of a medicament.

72 A medicament composition comprising an immunoglobulin according to claim 68 or claim 69 and a pharmaceutically acceptable excipient.